Bacterial Symbionts of Soft Coral Lobophytum sp from Panjang Island, Jepara, Indonesia with Antimicrobial MDR TB Potency

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BACTERIAL SYMBIONTS OF SOFT CORAL *LOBOPHYTUM* SP. FROM PANJANG ISLAND, JEPARA, INDONESIA WITH ANTIMICROBIAL MDR TB POTENCY

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ABSTRACT

Tuberculosis is a disease that attacks the lungs. The disease is caused by the bacterium *Mycobacterium tuberculosis*. The bacteria *M. tuberculosis* can be killed by antibiotics. However, continuous use of antibiotics can cause bacterial resistance. This study aims to determine the antibacterial activity of MDR TB from soft coral symbiont bacteria *Lobophytum* sp. The sample was collected and identification for colony morphology. An antibacterial preliminary test is conducting using the overlay method. Secondary metabolites extraction from bacterial was assayed for antibacterial activity. Bacterial symbiont was conducted for genomic DNA for molecular identification. There were six bacterial isolates obtained from soft coral *Lobophytum* sp. One isolate from *Lobophytum*-associated bacteria was successfully screened for antimycobacterial against MDR TB bacteria. PLO2 was found to inhibit the growth of MDR TB (MDR TB strain SIRE and R). Based on the results of identification with PCR, soft coral symbionts of PLO2 was closely related to *Virgibacillus marismortui* with homology of 99%.

Keywords: bacterial symbiont, *Lobophytum* sp., MDR TB, *Virgibacillus marismortui*

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INTRODUCTION

Several years ago, there was high confidence about the decline in defense of infectious diseases because of advances in technology and science. However, due to an increase in the transitional nature of infectious diseases, such beliefs have not been met. Worldwide infectious diseases information is still the leading cause of death, especially in developing countries,

claiming millions of lives yearly despite the enormous *improvements* made in human healthcare [1]. Treatment of infectious diseases has faced a major problem with a growing number of microbes developing mechanisms for antibiotic resistance and widely used antiviral therapy [2]. A few examples are the pathogens associated with and multidrug-resistant tuberculosis (MDR TB).

Tuberculosis is a highly contagious disease with about one-third of the world's sufferers. However, this problem is serious because *Mycobacterium tuberculosis* has developed a resistance mechanism for first-line treatment as well as second-line drugs. This has led to the emergence of *M. tuberculosis* (MDR), which is resistant to multiple drugs (MDR) and extensive drug resistance (XDR) throughout the world. Thus, overcoming the problem of infectious diseases is now an essential and urgent requirement.

Organisms in the sea including coral reef ecosystems have become a source of natural chemical products that are very interesting because they provide most of the sources of bioactive metabolite compounds with various biological activities [3]. The development of marine organisms derived compounds into drugs has been hampered by supply limitations [4].Symbiosis between microorganisms and marine organisms is abundant and widespread in the oceans [5] There is evidence that many of the previously identified marine soft coral secondary metabolites are of microbial origin [6]. Their ability to survive in this competitive environment could be due to their own adaptation, in addition to or perhaps because of the microbial communities be due to their adaptation, in addition to or perhaps because of the microbial communities, they harbor [7].

The ability of the soft coral in the bioactive compound due to the symbiotic relationship with microorganisms in this bacteria. This relationship includes the provision of nutrition by helping translocations metabolism including nitrification, nitrogen fixation, photosynthesis and helps chemical defenses as well as play a role in biofouling. Because of this the role of bacterial symbiotes then soft coral to have a high potential in generating bioactive compounds that had been isolated from the soft coral [8]. This potential allows symbiotic bacteria in the soft coral to produce bioactive compounds and replace soft coral that has produced bioactive compounds. Bacteria are capable of producing rapidly bimossa content so that bioactive lives can be produced more easily, quickly and more in the scale of biotechnology than in soft coral culture itself [9].

MATERIALS AND METHOD

Sample Preparation

Sampling and isolation bacterial symbionts Colonies of soft coral *Lobophytum* sp. were collected by scuba diving from Panjang islands, Jepara, North Java Sea, Indonesia (Figure 1).

Upon collection, soft corals were put into sterile plastic bags (Whirl-Pak, Nasco USA). The tissue was then rinsed with sterile seawater and homogenized with a blender. The homogenized tissues were serially diluted, spread on half strength ZoBell 2216E marine agar medium and incubated at room temperature for 2×24 hours. By morphological features, colonies were randomly picked and purified by making streak plates [10]. Purification was conducted by separation of colony morphology (color, shapes, margin, and texture).

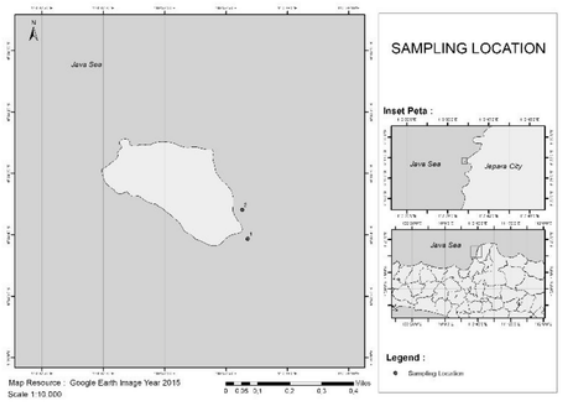


Figure 1. Sampling location of *Lobophytum* sp.

Antibacterial preliminary test

MDR TB strain SIRE (*Mycobacterium tuberculosis*) resistant Streptomycin, Isoniazid, Rifampicin and Ethambutol) and strain R (*Mycobacterium tuberculosis* resistant Rifampicin) was obtained from the Health Laboratory of Central Java Province-Semarang. This test was conducted using the overlay method [11]. The culture of each bacterium in the logarithmic phase was mixed with Middle brook 7H9+OADC soft agar medium (1% v/v), which were poured on to the respective agar surface previously inoculated with *Lobophytum* sp. associated bacteria that incubated for four days at room temperature. Then the plates were incubated at room temperature 2×24 hours. Inhibition zone showed that bacterium produced potential bioactive compound against MDR-TB. Furthermore only the most potential isolate was extracted of its secondary metabolites

Secondary Metabolites Extraction of Potential Isolate

Extraction was initially started with growing the associated bacterium into Zobell agar medium. Collected pellets, and the pellet was extraction using methanol with the aid of a sonicator. The crude extract was filtered using filter paper Whatman no. 1. Afterward, extract was then dried by the use of rotary evaporator and nitrogen (N_2) gas flow [12].

Antibacterial Test

This test was performed using the disk diffusion method. The crude extract from potential isolate was tested in various concentration. The concentration started from 10, 20, 30, 40, and 50 %. An inoculum of MDR TB strain SIRE and strain R with density of 0.5 McFarland in suspended with Middle brook 7H9+OADC soft agar medium (1% v/v). Crude extracts were on the blank paper disc, then incubated for 2x24 hours at room temperature. Antibacterial activity was the formation of clear zones around the MDR TB bacterial colonies [11].

Phytochemical Screening

Crude extract was evaluated by phytochemical qualitative reactions for secondary metabolites. The screening was performed for phenol, terpenoid, alkaloids, steroids, flavonoids, saponins, tannins, and triterpenoid. The color intensity or the

precipitate formation was used as analytical responses to these tests [13-15].

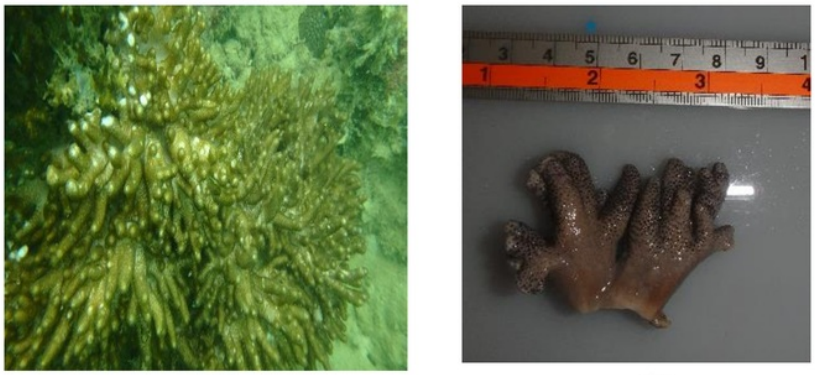
Molecular Identification

The genomic DNA of bacterial symbiont was conducted using chelex [16]. Amplification was done using PCR with Eubacteria universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3'), and primer 1492 (5'-TACGGYTACCTTGTTACGACTT-3') [17]. The proses of denaturation were initially at 94°C for 2 minutes and then successive denaturation (94 °C for 1 min), annealing (55 °C for 1 min), and extension (72 °C for 2 min). A series of denaturation, annealing, and extension were repeated 45 times. Furthermore, the gel electrophoresis was conducted to see the DNA bands formed. PCR amplification or partialm16S rRNA gen of *Lobophytum* sp. associated bacteria and subsequent sequencing analysis were performed according to a method of Altschul [18] and Radjasa [19].

RESULT AND DISCUSSION

Identification of *Lobophytum* sp.

Lobophytum sp. sample used in this research ware take from Panjang Island, Jepara (Figure 2). The morphology identification is shown in table 1. Softcoral genus *Lobophytum* sp. have several secondary metabolites, such as alkaloids, steroids, triterpenoids, flavonoids, saponins, phenols and terpenoids [20]. Terpenoids from *Lobophytum* sp. have a potential antibacterial activity[20, 21].



(a) (b)

Figure 2. *Lobophytum* sp. (a) in the sea and (b) on land

Table 1. The morphology identification bacteria symbiont from soft coral *Lobophytum* sp.

No	Bacterial Code	Coulour	Shape	Margin	Elevation
1.	PLO1	White	Irregular	Undulated	Curved
2.	PLO2	Milk White	Irregular	Undulated	Flat
3.	PLO3	Yellow	Circular	Intact	Flat
4.	PLO4	Yelow	Irregular	Undulated	Flat
5.	PLO5	White	Dots	Intact	Flat
6	PLO6	White	Circular	Intact	Curved

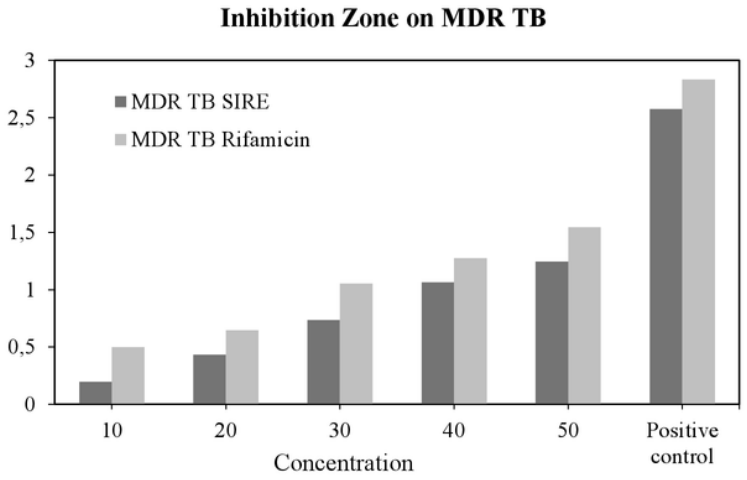


Figure 3. Bacteria activity test

Table 3. Phytochemical screening secondary metabolite from bacterial symbiont PLO2

No	Secondary metabolite	Explanation
1.	Phenol	-
2.	Tannins	-
3.	Alkaloids	+
4.	Steroids	-
5.	Flavonoids	-
6.	Saponins	-
7.	Terpenoid	+
8.	Triterpenoid	+

There was six isolates from symbiont bacteria soft coral *Lobophytum* sp. The result confirmed that marine organism such as *Lobophytum* sp. thrive in symbiosis with other microorganisms (bacteria) [22]. Bacterial will be symbiotic with their host. There are several factors that influences the quality and quantity of bacterial symbionts in the host that changes temperature, climate, and intensity to sun exposure [23].

Antibacterial preliminary test

The overlay test MDR TB from bacteria symbiont *Lobophytum* sp. shown in table 2. Overlay rest result shows that of the six bacterial isolates there was one isolate showing activity against bacteria MDR TB. Multidrug-resistant tuberculosis (MDR TB) that used for this research were MDR TB strain SIRE, and MDR TB strain R. SIRE is the first line drugs to patient tuberculosis. Microorganisms associated with marine invertebrates producing secondary metabolites are bioactive compounds that have the potential as a drug and Pharmaceutical dosage [22, 23]. According to to [24] that bacterial symbionts of *Lobophytum* sp. have activity against MDR bacteria.

Antibacterial Test

The results of antibacterial activity test on symbiont bacteria extract against MDR bacteria are presented in Figure. 3. One isolate was chose because they have activity against bacteria MDR TB. Isolates PLO2 were tested in MDR TB with five different concentrations. Their potential antibacterial was from secondary metabolites microorganism that produced when bacteria cell finished the logarithmic phase and going into stationer phase. Secondary metabolites of microorganism can be derived from the conversion primary metabolite; this phase is called idiophase [25].

Phytochemical Screening

Phytochemical screening secondary metabolite from bacterial symbiont PLO2 shown in table 3. In the present study, crude extract from bacterial symbiont PLO2 showed positive results for alkaloids, terpenoid, and triterpenoid. Bacteria symbiotically associated with soft corals can synthesize secondary metabolites similar to host [26]. The antibacterial mechanism of alkaloids is attributed to their ability to intercalate with DNA. Moreover, the mechanism of terpenoid is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds [27].

Molecular Identification

PCR method used to dertemine to molecular identification bacteria symbiont with *Lobophytum* sp. [24]. DNA sequencing of 16S rDNA from PLO2 isolate:

GCGGCTGGCTCCAAAAGGTTACCTC
ACCGACTTCGGGTGTTACCAACTCT
CGTGGTGTGACGGGCGGTGTGTAC
AAGGCCCGGGAACGTATTCACCGC
GGCATGCTGATCCGCGATTACTAGC
GATTCCGGCTTCATGCAGGCGAGTT
GCAGCCTGCAATCCGAACTGAGAA
TGGTTTTATGGGATTTGCTTGACCT
CGCGGCTTCGCTGCCCTTTGTTCCA
TCCATTGTAGCACGTGTGTAGCCCA
GGTCATAAGGGGCATGATGATTTG
ACGTCATCCCCACCTTCCTCCGGTT
TGTCACCGGCAGTCACCTTAGAGTG
CCCAACTAAATGCNGGCAACTAAG
ATCAAGGGTTGCGCTCGTTGCGGGA
CTTAACCCAACATCTCACGACACGA
GCTGACNACAACCATGCACCACCT
GTCACTCTGTCCCCCAAGGGAACAT
CCTATCTCTAGGATTGGCAGAGGAT
GTCAAGACCTGGTAAGGTTCTTCGC
GTTGCTTCG

Below is a bacterial symbiont homology search using the BLAST system, presented in table 4. And phylogenic tree shown in Figure 4. Phylogenic tree shown the phylogenetic affiliation of bacterial isolate with othe microorganism.

Table 4. Symbionts Bacteria Homology Search Results Using BLAST

No	Isolate Code	Nukleotide Length (bp)	Closest relative	*Accession Number*	Homology (%)
1.	P.Lo 2	502	*Virgibacillus marismortui*	NR_028873	99

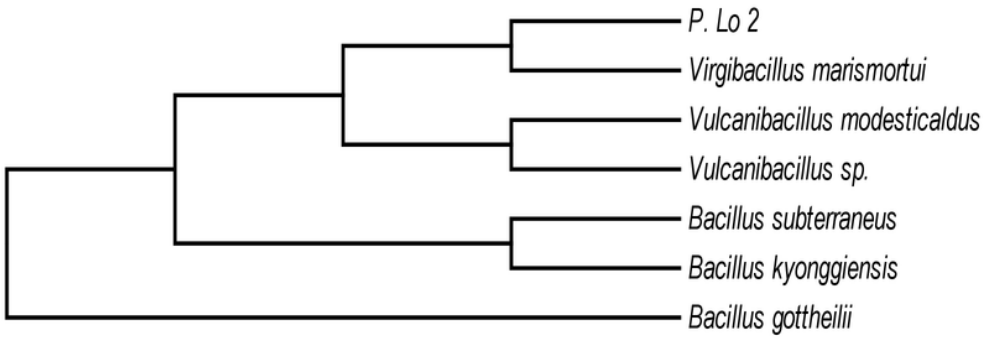


Figure 4. Phylogenic tree from bacterial simbionts PLO2 from *Lobophytum* sp.

Molecular identification using the BLAST system, by search for the bacterial homology. Isolate PLO2 was strictly related to *Virgibacillus marismortui* with 99% homology value. [28, 29] state that isolates which has 16S rDNA sequence similarity more than 97% can be represented at the species level. While the sequence similarities between 93 – 97% can represent identity at the genus level. It can be concluded that PLO2 have a level similarity in species. *Virgibacillus marismortui* is a species of Gram-positive, it can be obligate aerobs. [30, 31] found that bacterial *Virgibacillus marismortui* from Dead Sea water and deteriorated mural paintings.

CONCLUSION

PLO2 symbiotic bacteria from Lobophytum sp have antibacterial activity against MDR TB strain SIRE and strain R. Isolate PLO2 identified as *Virgibacillus marismortui* based on accesion NR_028873

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